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Characteristics of spermatozoa and reproductive organs in relation to age and body weight in Swedish moose (*Alces alces*)

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ABSTRACT

Knowledge of the reproductive biology of game species is vital for sustainable management. In moose (*Alces alces*), research in reproductive characteristics has focused on the female, whereas there are few studies in male moose. The aim of the present study was to investigate sperm morphology and chromatin integrity (SCSA), and their relationships with testicular and epididymal features, as well as temporal aspects with respect to the hunting season. In total, 143 male moose aged 1.5–11.5 years were sampled from 2008 to 2011. The proportion of normal spermatozoa (PNS) ranged from 1.5% to 82.0%, with a mean of 51%, and the %DFI (DNA fragmentation index) ranged from 2.5% to 36.7% (mean 9.5). PNS decreased temporally, and was positively associated with carcass and testes weight. Body weight and testes weight had positive effect on PNS regardless of age. No effect of any explanatory variables was observed on the DFI. The testis/body weight ratio of moose (0.033%) is among the lowest reported among mammals, indicating a less polygynous mating system than in roe deer and red deer. For reproduction success in moose, a high body weight in males is favorable, as is a balanced sex ratio. Thus, males should not be harvested prior to the time when the majority of females have passed their first oestrus of the season.

1. Introduction

A keystone game species of the circumpolar areas of the northern hemisphere is the moose (*Alces alces*), which is an important source of food, recreation, and tourism (Boman et al., 2011). In Fennoscandia (Finland, Norway, and Sweden) about 25% (200,000 animals) of the estimated population is harvested annually (Lavsund et al., 2003; Nilsen and Solberg, 2006; Pusenius et al., 2008). Successful management of a game species relies on knowledge about basic ecology, in which reproduction is crucial and the driver of population sustainability. To enable high harvest numbers in relation to the population size, a high reproductive output is required. In addition

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to harvest, reproduction is also affected by population density, mortality rates, forage availability, and climate (Gaillard et al., 2000; Solberg et al., 1999).

Research on moose reproduction has mostly focused on female characteristics such as age and weight at sexual maturity (Ferguson, 2002; Sand and Cederlund, 1996; Sæther and Heim, 1993), fecundity at different ages (Ericsson et al., 2001; Garel et al., 2009; Sand et al., 1996; Solberg et al., 2007; Sæther and Haagenrud, 1983), and senescence (Ericsson et al., 2001; Garel et al., 2009), whereas studies on male moose reproductive characteristics are relatively few.

During the rut, males increase their activity in search of females in oestrus, resulting in weight loss (age-dependent reproductive effort), which is most prominent in males aged 6–12 years (Mysterud, 2005). According to Solberg and Sæther (1994), body weight and antler size are characteristics that are important for mating success of moose males. The pubertal development is a gradual process where the sperm production and quality, libido, and mating ability increase with age (Lunstra et al., 1978). Apart from reports that moose bulls may reach puberty as yearlings and can mate successfully (Bubenik and Timmermann, 1982; Schwartz et al., 1982; Schwartz, 1992), most parameters of male moose reproduction have not been fully documented.

The relative testes size (in relation to body weight) in mammals is indicative of the degree of polygyny, i.e. the mating of one male with several females, and larger testes generally reflect that a male can cover several females during a limited period of time (Clutton-Brock and Guinness, 1982; Kenagy and Trombulak, 1986). Thus, as stated by Short (1997) 'the testis is the witness of the mating system.' Taiga-living moose, such as the European subspecies, show a more monogamous mating behavior compared to tundra-living moose that form assemblages of females during the rut (Schwartz, 2007). In taiga moose, a skewed sex ratio in favor of moose females may affect the reproductive output by delaying the time of mating that, in turn, delays the time of calving (Solberg et al., 2002). In addition, Schwartz (2007) suggested that moose have small semen reserves and thus are not able to breed many cows in a short time. Thus, the testis/body weight ratio in moose males is of interest in order to attain understanding of the reproductive biology and mating system of the species.

Based on histological examinations of testes from moose, Bubenik and Timmermann (1982) assessed at what age the onset of sperm production occurred, as well as the seasonal pattern of the sperm production in young males. Similar studies, based on smaller sample sizes, were conducted by Peek (1962), and Houston (1968) in North American moose, which along with the results of Bubenik and Timmermann (1982), showed that some bulls reach puberty as yearlings, and that the occurrence of puberty increased with age. However, in order to establish the age when moose become pubertal, additional criteria are required. In domestic animal breeding, semen concentration and volume (total number of spermatozoa per ejaculate), sperm motility, freezability (for artificial insemination), and fertility result after insemination are used as important parameters to evaluate overall sperm characteristics and reproductive capacity. In wildlife, most of these parameters are difficult to assess although some of them (sperm morphology and motility, chromatin integrity, freezability, and sperm concentration) have been evaluated in roe deer (*Capreolus capreolus*, Blottner et al., 1996; Goeritz et al., 2003), and red deer (*Cervus elaphus*, Martínez et al., 2008). Investigations of such reproductive parameters have so far not been performed in male moose, probably because of logistic difficulties since moose typically occur in low numbers in remote taiga or tundra habitat. However, in southern Sweden moose populations are dense, harvest numbers are high, and hunting areas are accessible which enable sampling and instant field investigation of reproductive organs from wild moose.

To increase knowledge on moose reproductive biology in general and male moose reproduction in particular, the aims of the present study were (a) to investigate sperm quality (morphology and chromatin integrity) in relation to age, weight, and testicular features (weight of testes and epididymides); (b) to investigate temporal aspects on sperm quality (seasonality); and (c) to assess basic information (weights of testes, epididymides, and the testes/body weight ratio) of male reproductive organs in Swedish moose during the hunting period. The results are important to establish harvest principles for sustainable moose management.

2. Materials and methods

2.1. Study area and sampling of harvested moose

Reproductive organs (penes, testes, epididymides, and accessory glands) and mandibles were collected by field personnel from hunter-harvested male moose in Sweden, south of the 59th latitude and north of the 56th latitude (Fig. 1), during the first month of the hunting period (Oct. 10–Nov. 8) from 2008 to 2011. Individual identification numbers were assigned to each specimen and adjoining samples. Date, place, time of harvest, and time of sample collection were noted. In order to facilitate the handling of fresh samples, temporary field laboratories were set up in the study area during the sampling periods each year. Collected samples were transported to the laboratories in coolers (at approximately +8 °C). Carcass weights head, skin, internal organs, and metapodials removed, (Wallin, 1996) was recorded by the hunters and reported to the researchers. Body weight was calculated according to Markgren (1969), and Wallin (1996) and combined testes weight were used to calculate the testis/body weight ratio.

2.2. Age determination

For age determination in adult moose, the first molar of the lower jaw was sectioned and cementum layers counted as described by Wolfe (1969). Prime-aged males (6–12 years of age) followed the classification according to Mysterud et al. (2005).

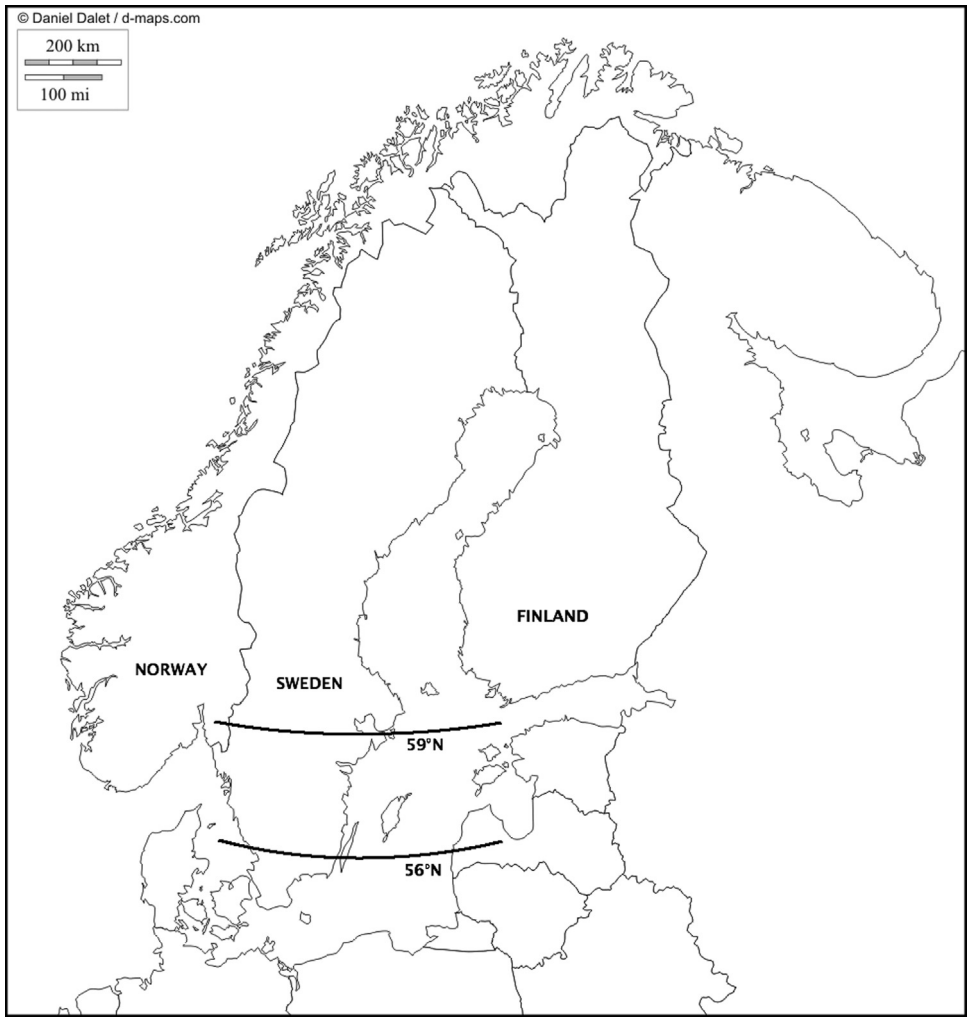

Fig. 1. Geographical location of moose bulls sampled in southern Sweden from 2008 to 2011.

2.3. Sample examination and preparation in field laboratory

On arrival at the field laboratory, time was noted, testes were removed from the scrotum, and the epididymis was separated from each testis. Epididymides and testes were weighed and measured. From each of the cauda epididymis, a 10 mm incision was made and three sperm samples of approximately 0.1 ml each were collected with a sterile plastic 1-ml pipette (Copan Italia, Brescia, Italy). Of the three aliquots, one was smeared on a glass slide (for wet mount sperm morphology), the second was fixed in formol–saline for preparation of dry smears (sperm morphology), and the third (from the left cauda only) was put in a 2 ml cryovial (BioCision, Larkson, USA) containing 0.5 ml TNE buffer (Tris–NaCL–ethylenediaminetetraactic acid) solution (50 mM Tris of pH 7.5, 140 mM NaCl, and 5 mM EDTA) for chromatin integrity analysis (SCSA). After placement in TNE buffer solution, the vial was immediately plunged into liquid nitrogen (−196 °C) and was subsequently transported in a dry shipper to the semen lab at the Swedish University of Agricultural Sciences, Uppsala, where the vials were stored in a −80 °C freezer until analyzed. The prepared glass slides and the formol–saline fixed samples were stored in room temperature until sperm morphology assessment.

Both testes were dissected and the cut surface inspected for the presence of macroscopic abnormalities or lesions such as spermiostasis or signs of orchitis. The texture (soft, normal, or hard) was evaluated by palpation of approximately 1 cm thick slices of the tissue.

2.4. Sperm morphology

Sperm head morphology was assessed in dry smears stained with carbol-fuchsin according to the method described by Williams (1920) and modified by Lagerlöf (1934). Five hundred spermatozoa (standard procedure) were assessed in each dry smear using phase contrast microscopy (1000×). The presence of proximal cytoplasmic droplets, abnormal acrosomes, detached heads and abnormalities of the mid-piece and tail were recorded. In the

wet mount preparation, 200 spermatozoa were assessed using phase-contrast microscopy (1000×) and classified according to the system developed by Bane (1961). Morphological abnormalities were recorded as the percentage of the total number of counted spermatozoa. An average of the samples from the left and right epididymis of each male was calculated for the different sperm morphological parameters. Furthermore, an average of the proportion of normal spermatozoa (PNS) for each male was calculated. All morphological evaluations of spermatozoa were performed by the same highly skilled technician, who has a very long experience of evaluating sperm samples from different animal species, including wildlife and zoo animals. In accordance with studies performed in roe deer (Goeritz et al., 2003) and red deer (Malo et al., 2005), cut-off values for acceptable proportions of normal spermatozoa were set at ≥70%. This corresponds with cut-off values used in dairy and beef bulls (Hopkins and Spitzer, 1997).

2.5. Chromatin integrity analysis (SCSA)

The procedure for the SCSA followed the protocol originally developed by Evenson et al. (1980) and later described in detail by Evenson and Jost (2000). Abnormal chromatin structure was defined as the susceptibility of sperm DNA to undergo acid-induced denaturation in situ. Following exposure of the prepared DNA toacridine orange (AO), the degree of chromatin integrity was analyzed by flow cytometric measurement (FCM) of the metachromatic shift from green (stable, double-stranded DNA) to red (denatured, single-stranded DNA) AO fluorescence (Evenson et al., 1980). This shift was expressed as the ratio of red to total (i.e. red and green) fluorescence intensity. In the SCSA, this ratio was calculated for each spermatozoon within a sample and the results were expressed as the ratio of single-stranded to double-stranded DNA (DFI, Evenson et al., 2002).

A sample from a domestic cattle (*Bos taurus*) with known high chromatin instability were processed in the same manner and served as control for the procedure. Just prior to analysis, the samples were thawed on crushed ice. The thawed, TNE-extended spermatozoa were subjected to partial DNA denaturation in situ (by mixing with 0.4 ml of a low pH detergent solution containing 0.17% Triton X-100, 0.15 M NaCl, and 0.08 N HCl, pH 1.2), followed 30 s later by staining with 1.2 ml of AO (6 μg/ml in 0.1 M citric acid, 0.2 M Na_2HPO_4, 1 mM EDTA, and 0.15 M NaCL, pH 6.0). The stained samples were analyzed within 3–5 min of AO staining. A sample from a domestic bull with known high chromatin instability was processed in the same manner and served as control for the procedure (reference sample for SCSA). Measurements were made on a FACStar Plus flow cytometer (Becton Dickinson, San José, CA, USA) equipped with standard optics. Acridine orange was excited with an Ar (argon) ion laser (Innova 90; Coherent, Santa Clara, CA, USA) at 488 nm, running at 200 mW. As previously mentioned, in association with double-stranded DNA, AO fluoresces green (530 ± 30 nm, as detected using the FL 1 detector), but in the presence of single-stranded DNA the fluorescence is red (>630 nm, as detected with the FL 3 detector). The fluorescence stability of the flow cytometer was monitored daily using standard beads (Fluoresbrite plain YG 1.0 μM; Polysciences Inc., Warrington, PA, USA). Equivalent instrument settings were used for all samples.

From each sample a total of 10,000 events were measured at a flow rate of ∼200 cells/s. Data collection was carried out using CellQuest, version 3.3 (Becton Dickinson, San José, CA, USA). Further calculations were performed using FCS Express version 2 (De Novo Software, Thornhill, Ontario, Canada).

2.6. Statistical analyses

The effect of explanatory variables (age, carcass weight, weight of testes and epididymides, day of sampling, and time elapsed from harvest to when the sperm sample was put in liquid nitrogen) on the response variables (chromatin integrity, %DFI, and proportion of normal spermatozoa, PNS) was calculated using logistic regression in R (R Core team, 2013). The association between carcass weight, testes weight and proportion of normal spermatozoa was tested with a linear regression in R (R Core team, 2013).

3. Results

3.1. Sampled moose

In total, 143 moose males were sampled during the hunting period for four consecutive years (2008–2011). However, depending on different circumstances (e.g. failure to report carcass weight, mistakes during the hunter's evisceration of internal organs and/or removal of mandibles prior to sampling of moose) it was not possible to record all parameters in all specimens. The mean time elapsed from harvest to field laboratory examination was 6.7 h (range 1–24 h). The mean age of the sampled moose was 3.9 (range 1.5–11.5 years). Carcass weight was recorded in 67.1% ($n = 96$) moose, with a mean of 192.0 kg (range 75–302 kg, Fig. 2), which corresponds to a calculated mean live weight of 349.1 kg (range 136.4–549.0 kg). A total of 28 prime-aged males were sampled and included in the present study (19.6% of all sampled males).

3.2. Weight of testes and epididymides

Complete sets of testes and epididymides were collected from 93.3% ($n = 133$) of all age-determined individuals. The mean weight of the left and right testis was 55.7 g (range 8.6–87.1 g), and 56.3 g (range 9.3–118.6 g), respectively, and for the left and right epididymis 11.0 g (range 2.0–19.0 g) and 10.7 g (range 2.7–18.4 g), respectively (Fig. 2). There was no difference ($p > 0.05$) between the weights of the left and right testis and the left and right epididymis, respectively. There was a linear relationship ($R^2 = 0.45$ and 0.39, respectively) between testes weight and carcass weight (two subsets of samples depending on time of sampling used, Fig. 3). The mean testes weight was higher during the first part of the sampling period (Oct. 10–Oct. 16) than during the last part (Oct. 30–Nov. 8). The mean testes/body weight ratio ($n = 96$) was 0.033% (SD ± 8.0×10^{-5}, range 0.008–0.051%).

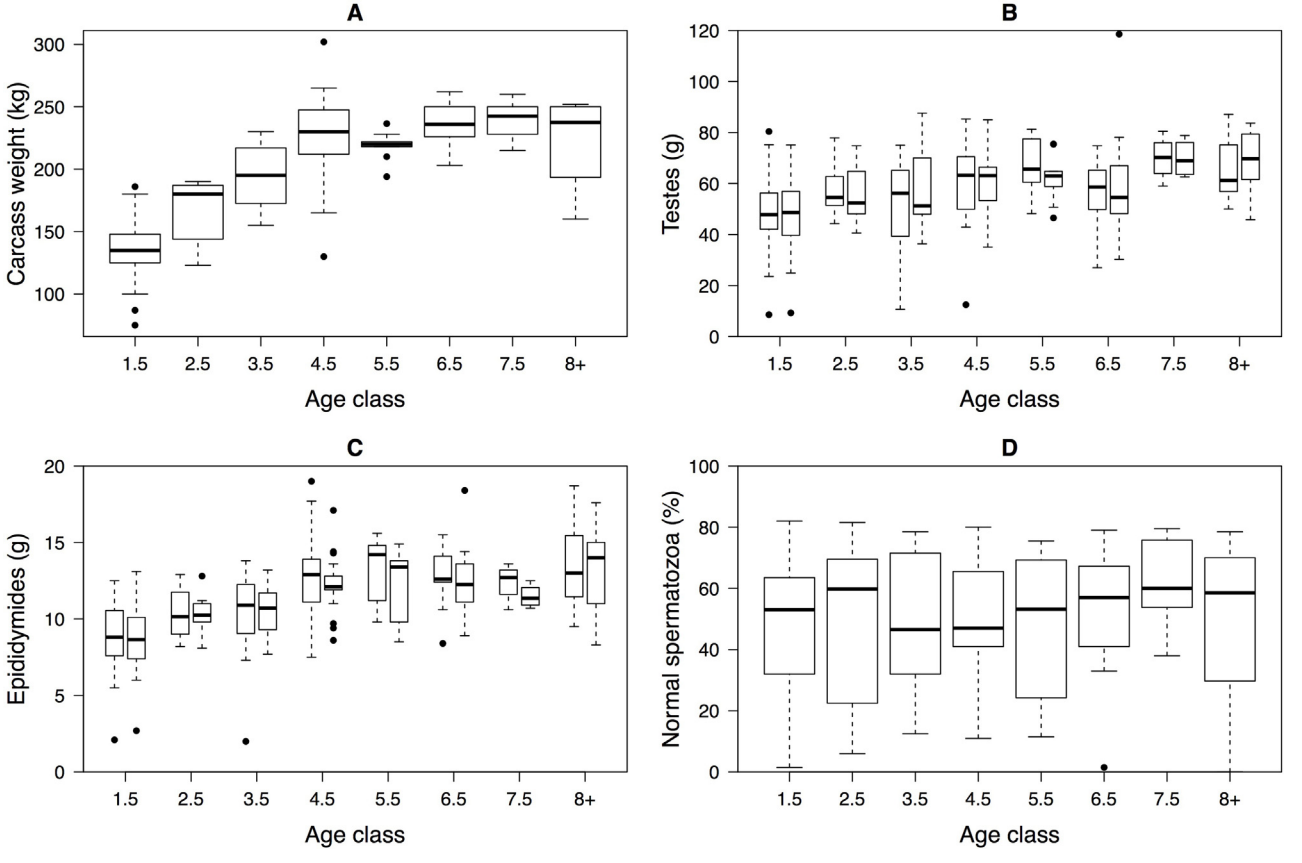

Fig. 2. Box and whisker plots of the relationship between age group and carcass weight (A), left and right testes weight (B), left and right epididymides weight (C), and proportion of normal spermatozoa (D) of harvested moose bulls in southern Sweden sampled from 2008 to 2011.

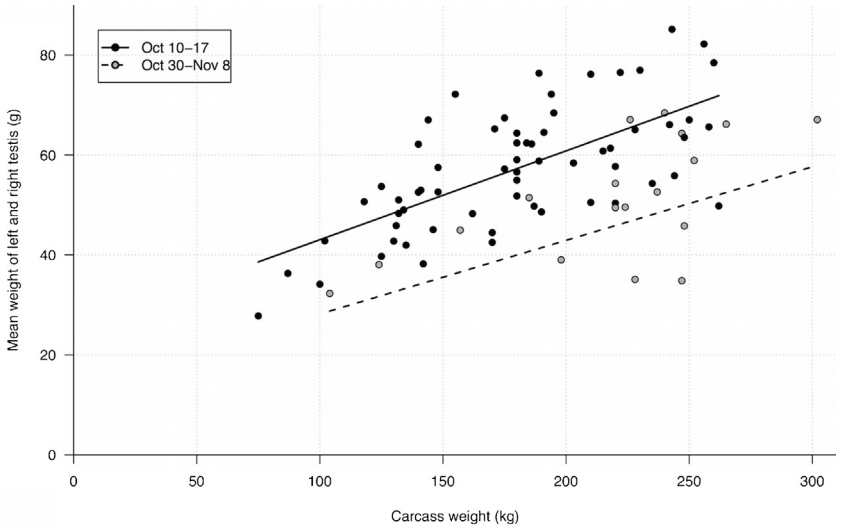

Fig. 3. Linear associations between carcass weight and total testes weight for two different sampling periods (Oct. 10–17, $n = 63$ and Oct. 30–Nov. 8, $n = 18$) in Swedish moose bulls.

Cryptorchidism (undescended and missing testis) was confirmed in three males (2.2%). In one of these males, one testis (36.0 g) was found in the abdomen while the other testis was located in the scrotum and was much larger (118.6 g). In another three cases, one of the testes was missing but the reason behind this is unclear, except for in a fourth case where, one testis was observed being taken by a raven (*Corvus corax*) just after evisceration.

3.3. Sperm morphology

Sperm morphology assessments were performed in samples from 124 age-determined moose bulls with complete sets of testes and epididymides. The primary parameter used for sperm quality was the proportion of normal spermatozoa (PNS). The mean PNS for all sampled bulls was 51.0%, (range 1.5–82.0%, Figs. 2 and 6). From October 10–16, the mean PNS was 55.1% ($n = 100$, range 1.5–82.0%). From October 30 to November 7, the corresponding PNS was 33.2% ($n = 24$, range 0–64.5%), which differed ($p < 0.01$) from the previous period. There was a significant negative effect ($p < 0.05$) of time of sampling on the PNS (Fig. 4) whereas no effect of age, sampling year, or time elapsed between harvest and arrival to field laboratory was observed. Furthermore, there was a positive effect ($p < 0.05$) of testes weight (and carcass weight) on the PNS, regardless of time of sampling, sampling year, or age (Fig. 5). Sperm assessment results from the age-determined bulls are presented in Fig. 6. Moose of all ages displayed varying PNS. Yearlings (1.5 years of age) and bulls aged 2.5 years had significantly higher ($p = 0.047$) proportions of proximal cytoplasmic droplets than older males regardless of time of sampling. No differences ($p = 0.91$) in proportions of abnormal sperm heads between young (1.5–2.5 years of age) and older males were observed (Fig. 6). Of all animals, 29 individuals (24.4%) had a PNS exceeding 70%, 55 individuals (44.4%) had a PNS exceeding 60%, and 72 individuals (58.0%) had a PNS exceeding 50%.

3.4. Sperm chromatin analyses

Chromatin analyses were performed in 104 age-determined bulls, and a SCSA cytogram is presented in Fig. 7. The mean, and median DNA fragmentation index (DFI) was 9.5%, and 8.8% (range 2.5–36.7%, Fig. 6), respectively. There was no significant effect of any of the explanatory variables on the %DFI.

4. Discussion

In this study, we describe sperm morphology and chromatin integrity, and their relationships to male characteristics in moose over the course of the first month of the hunting period in Sweden. We found that carcass weight and testes weight, regardless of age, was positively associated with the proportion of normal spermatozoa and that the morphological quality (PNS) decreased significantly from the beginning/middle of October to the beginning of November. Furthermore, the majority of the bulls had lower proportions of normal spermatozoa than the set cut-off value. These results combined indicate that a high body weight, rather than age, is important for reproductive success in moose bulls, and that the breeding season for bulls is nearing its end in the middle of November. In addition, the testes:body weight ratio indicated that moose are not anatomically adapted for a polygynous mating system. To our knowledge, this is the first report on sperm morphology in moose using a large and well-defined material.

In the present study, sperm morphology and chromatin integrity were evaluated in the light of results from studies in red deer and roe deer, since there are few reports from studies of moose. However, the mating systems of these species have to be considered, as red deer hold harems (Clutton-Brock and Guinness, 1982), whereas roe deer (Liberg et al., 1998) and Eurasian (taiga-living) moose do not (Markgren, 1969).

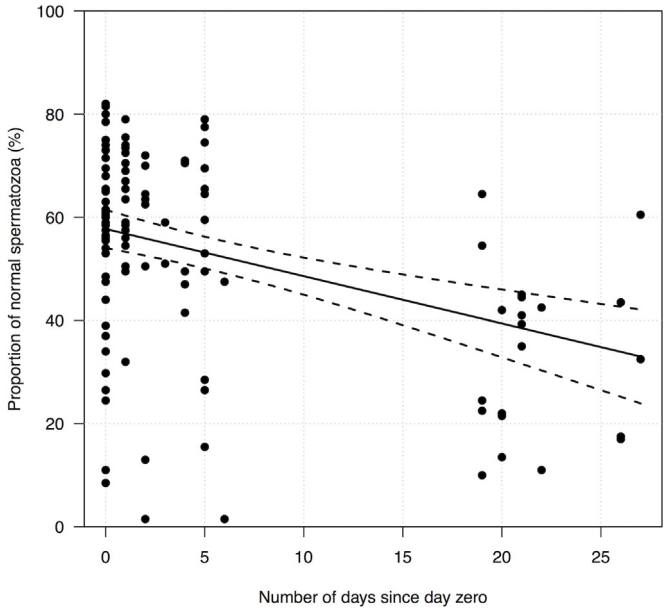

Fig. 4. The relationship between proportion of normal spermatozoa and number of days since the start of the hunting period in moose from southern Sweden.

In the present study, the spermatozoa were collected from the caudae epididymis. The time from harvest of the reproductive organs to sampling of spermatozoa at the field laboratory seemingly did not affect the spermatozoa, most likely due to the fact that the time from sampling until analysis did not exceed 24 h. In red deer, Soler et al. (2005) reported acrosome integrity to be negatively affected 48 h after sampling. In addition, red deer sperm motility reportedly is affected by time elapsing from sampling until analysis (Martínez-Pastor et al., 2005), but this parameter was not evaluated in the moose sperm samples in the present study. Furthermore, it has been shown in stallions that storing epididymides in 5 °C for 24 h does not negatively affect spermatozoa (Bruemmer et al., 2002; James et al., 2002). The proportion of normal spermatozoa decreased from mid-October to the beginning of November in the present study, which corresponds to the reported reproductive behavioral seasonality in male moose (Cederlund and Sand, 1994; Markgren, 1969; Miquelle, 1990; Sigouin et al., 1995; Van Ballenberghe and

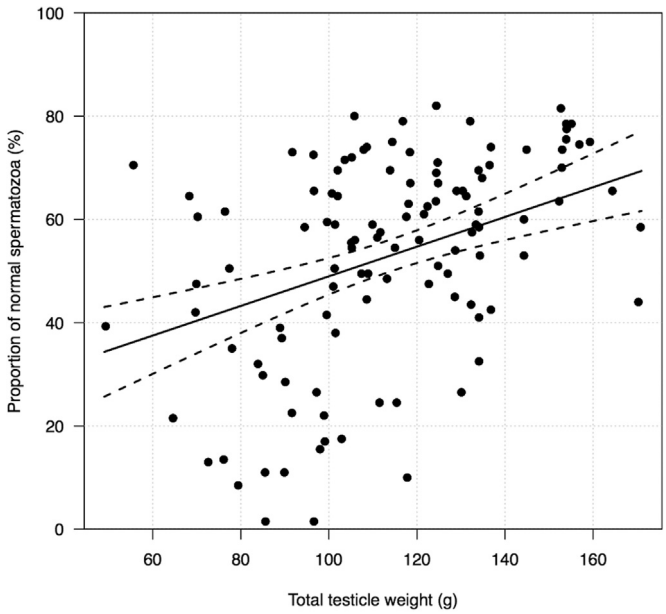

Fig. 5. The linear relationship between mean testes weight and proportion of normal spermatozoa in harvested moose bulls ($n = 125$) from southern Sweden.

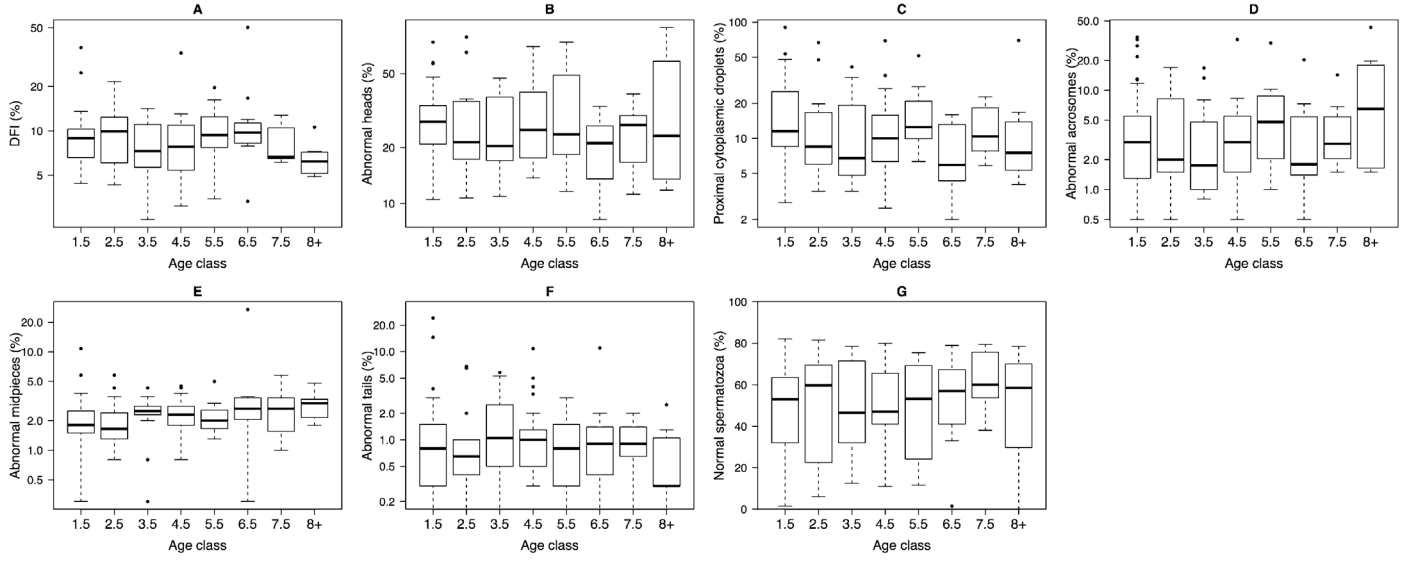

Fig. 6. Box and whisker plots of the relationship between age groups and chromatin integrity (DFI, A) and sperm morphology parameters (B–G) in epididymal sperm samples from 124 harvested and age-determined moose bulls in southern Sweden sampled from 2008 to 2011.

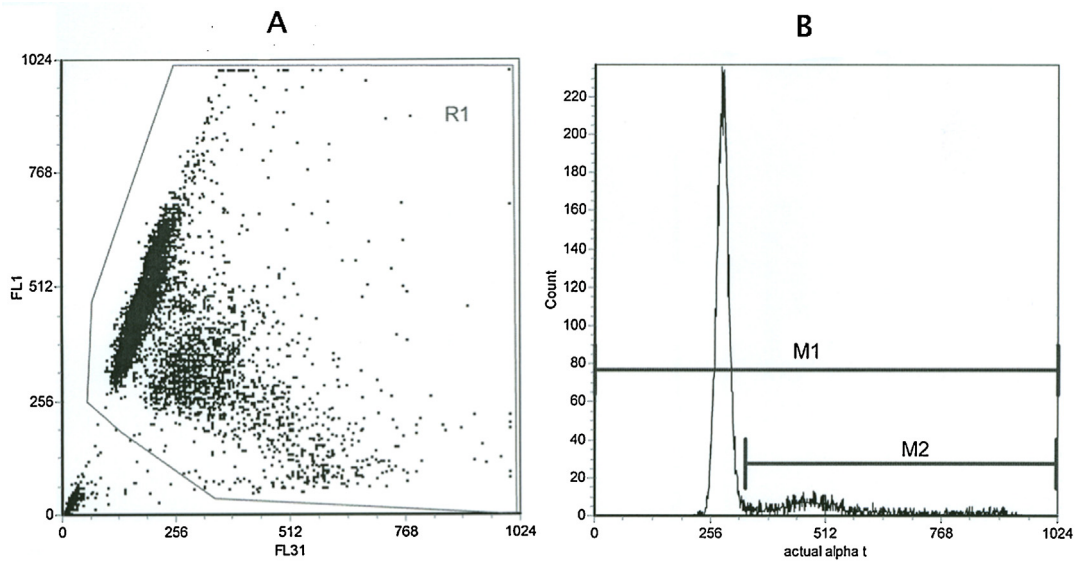

Fig. 7. Raw data from SCSA analysis of Swedish male moose. Fig. 7A shows a dot-plot of red fluorescence (*X*-axis) and green fluorescence (*Y*-axis). Cells in region R1 were further analyzed using the alpha-*t*-distribution shown in Fig. 7B. Cells in region M2 were considered to have a high ratio of denatured, single-stranded DNA.

Miquelle, 1993, 1996). Using the cut-of values of 70%, as for roe deer (Goeritz et al., 2003) and red deer (Malo et al., 2005), only a low proportion of the sampled moose had acceptable PNS during the sampling period. Further studies are needed to find out if this result is normal for moose or not.

A decline from 70% to 35% in normal spermatozoa was observed in captive roe deer (ejaculate samples) from August to September by Goeritz et al. (2003). Martínez-Pastor et al. (2005) reported similar findings for overall sperm quality in wild roe deer (epididymal sperm) from Spain. In red deer, rutting activity, testosterone production (Lincoln, 1971), and testes volume/weight (Hochereau-de Reviers and Lincoln, 1978; Pinsker, 1978) decreased as the mating season drew to an end. In domestic animals, such as stallions, a similar association between testes size and sperm production has been reported (Thompson et al., 1979). In the present study, the mean testes weight decreased over the time period studied, indicating that a decline in sperm production and quality also takes place in moose bulls, which in turn indicates that the peak rut is over by the time the hunting period commences. This is supported by the findings of the timing of oestrus in female moose, where most adult females show oestrus just prior to the start of the hunting period in moose in southern Sweden (Malmsten et al., 2014).

In domestic species, high incidences of cytoplasmic droplets and abnormal heads (Barth and Oko, 1991) indicate an immature semen picture in young males, but it can also be a sign of impaired sperm production caused by e.g. testicular degeneration. Testicular degeneration, which can be caused by increased body temperature, trauma, or an infection in the testes, may explain why some of the moose aged >3 years showed high incidences of abnormal sperm heads and proximal cytoplasmic droplets.

In the present study, no difference in the proportions of abnormal sperm heads was observed between young and old males, indicating that this parameter may not be indicative of puberty in moose, whereas the proportion of proximal cytoplasmic droplets was higher for young males. The high proportion of proximal cytoplasmic droplets in young males (1.5–2.5 years of age) resulted in a lower PNS, which along with lower carcass weights indicated that most of the males in this age category probably had not reached puberty. Nevertheless, young males with high carcass weights (and corresponding high testes weights) were found to have acceptable PNS (>70%), whereas old males with low carcass weights had lower PNS. Again, this indicates that the body weight is more important for a high PNS than age of the male. As a comparison, the time at which yearling females reach puberty is also related to body weight (Sæther and Haagenrud, 1983), rather than age (Malmsten et al., 2014). Since weight at birth in moose is negatively associated with adult body weight (Solberg et al., 2004), the birth weight may affect puberty in both males and females.

We found no significant association between age and testes weight, but testes weight was significantly correlated with body weight and in turn also with PNS. This differs from red deer, where age reportedly has a stronger effect on testes size and subsequently also quality of spermatozoa (Martínez et al., 2008). From a moose management perspective, this emphasizes the importance of having males with high body weights, and not necessarily old males in the population, in order to ensure that females are courted and mated by males with potentially better sperm morphology.

The testes/body weight ratio was considerably lower than reported in roe deer (0.22%) and red deer (0.18%, Kenagy and Trombulak, 1986) indicating that moose are to a much lesser extent anatomically adapted to mate several

times during a limited time period than some other species. It is not known how many females a male moose covers during the breeding season, as opposed to red deer stags, which mate several hinds (Clutton-Brock and Guinness, 1982; Guinness et al., 1971). The testes:body weight ratio in moose suggests that a balanced sex ratio of adult taiga moose is required in order to obtain high pregnancy rates, along with high body weights.

To optimize birth conditions for calves, the conception should take place during the first oestrus of the reproductive season. Calves are then born in May or the beginning of June the following year, at the prime of the forage (plant) growth season. Reproductive season in terms of the oestrus period, from the end of September to the beginning of October has been reported in moose (Garel et al., 2009; Haagenrud and Markgren, 1974; Markgren, 1969; Monfort et al., 1993; Schwartz and Hundertmark, 1993). However, a recent Swedish study (Malmsten et al., 2014) showed that some young adults, (or old cows with low body weight) did not have their first oestrus of the season until the end of October/beginning of November. The results of the present study indicate that mating during the latter part of October or beginning of November (during a late first oestrus or a second oestrus) may result in lower conception rates due to lower sperm quality since we found that the PNS decreased over time. This, in turn, may reduce the chance of fertilization.

The assessment of chromatin integrity analyses in the present study did not appear applicable for sperm quality assessment in moose. Chromatin integrity has previously been used for the evaluation of sperm quality in domestic animals. In stallions, assessment of chromatin integrity was reported to be associated with sperm quality based on morphological appearance (Morrell et al., 2008), but it does not correlate to the same extent in dairy bulls (Nagy et al., 2013). This may partly be due to a better stability of chromatin in ruminants than seems to be the case in stallions (Morrell, personal communication). Sperm chromatin has been investigated in red deer and is reportedly more condensed (lower %DFI) during the breeding season than during the non-breeding season (Garcia-Macias et al., 2006; García-Macías et al., 2006). Further studies are needed in moose to evaluate if the stability of chromatin integrity is the same as in domestic ruminants, and if there is seasonal variation.

In conclusion, based on sperm morphology, the present study showed a positive correlation between the carcass and testes weight and the proportion of normal spermatozoa in moose males. During the first month of the hunting period, the proportion of normal spermatozoa decreased over time. Moreover, moose males do not seem to be anatomically adapted for a high degree of polygyny. It is thus suggested that moose bulls with high body weight should not be hunted before the end of the normal oestrus period in females, and that a skewed sex ratio of the adult moose population should be avoided.

Conflict of interest

The authors declared that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anireprosci.2014.12.011.

References

Bane, A., 1961. Acrosomal abnormality associated with sterility in a boar. In: Proceedings of the Fourth International Congress on Animal Reproduction and AI, The Hague, pp. 810–817.
Barth, A.D., Oko, R.J., 1991. Abnormal morphology of bovine spermatozoa, first ed. Iowa State University Press, Ames.
Blottner, S., Hingst, O., Meyer, H., 1996. Seasonal spermatogenesis and testosterone production in roe deer (*Capreolus capreolus*). J. Reprod. Fertil. 108, 299–305.
Boman, M., Mattsson, L., Ericsson, G., Kriström, B., 2011. Moose hunting values in Sweden now and two decades ago: the Swedish hunters revisited. Environ. Resour. Econ. 50, 515–530.
Bruemmer, J.E., Reger, H., Zibinski, G., Squires, E.L., 2002. Effect of storage at 5 °C on the motility and cryopreservation of stallion epididymal spermatozoa. Theriogenology 58, 405–407.
Bubenik, A.B., Timmermann, H.R., 1982. Spermatogenesis in the taiga-moose of North-Central Ontario – A pilot study. Alces 18, 54–93.
Cederlund, G., Sand, H., 1994. Home-range size in relation to age and sex in moose. J. Mammal. 75, 1005–1012.
Clutton-Brock, T.H., Guinness, F.E., 1982. Red deer: behavior and ecology of two sexes, first ed. University of Chicago Press, Chicago.
Ericsson, G., Wallin, K., Ball, J.P., Broberg, M., 2001. Age-related reproductive effort and senescence in free-ranging moose, *Alces alces*. Ecology 82, 1613–1620.
Evenson, D.P., Darzynkiewicz, Z., Melamed, M.R., 1980. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 210, 1131–1133.
Evenson, D., Jost, L., 2000. Sperm chromatin structure assay is useful for fertility assessment. Methods Cell Sci. 22, 169–189.
Evenson, D.P., Larson, K.L., Jost, L.K., 2002. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J. Androl. 23, 25–43.
Ferguson, S.H., 2002. The effects of productivity and seasonality on life history: comparing age at maturity among moose (*Alces alces*) populations. Global Ecol. Biogeogr. 11, 303–312.
Gaillard, J.-M., Festa-Bianchet, M., Yoccoz, N., Loison, A., Toigo, C., 2000. Temporal variation in fitness components and population dynamics of large herbivores. Annu. Rev. Ecol. Syst. 31, 367–393.
Garcia-Macias, V., Martínez-Pastor, F., Alvarez, M., Garde, J.J., Anel, E., Anel, L., de Paz, P., 2006. Assessment of chromatin status (SCSA®) in epididymal and ejaculated sperm in Iberian red deer, ram and domestic dog. Theriogenology 66, 1921–1930.
García-Macías, V., Martínez-Pastor, F., Álvarez, M., Borragan, S., Chamorro, C.A., Soler, A.J., Anel, L., Paz, P., 2006. Seasonal changes in sperm chromatin condensation in ram (*Ovis aries*), Iberian red deer (*Cervus elaphus hispanicus*), and brown bear (*Ursus arctos*). J. Androl. 27, 837–846.

Garel, M., Solberg, E.J., Sæther, B.E., Grøtan, V., Tufto, J., Heim, M., 2009. Age size, and spatiotemporal variation in ovulation patterns of a seasonal breeder: the Norwegian moose (*Alces alces*). Am. Nat. 173, 89–104.
Goeritz, F., Quest, M., Wagener, A., Fassbender, M., Broich, A., Hildebrandt, T.B., Hofmann, R.R., Blottner, S., 2003. Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. Theriogenology 59, 1487–1502.
Guinness, F., Lincoln, G., Short, R., 1971. The reproductive cycle of the female red deer, *Cervus elaphus L.* J. Reprod. Fertil. 27, 427–438.
Haagenrud, H., Markgren, G., 1974. The timing of estrus in moose (*Alces alces L.*) in a district of Norway. Proc. XI Int. Congr. Game Biol. Stock. 1973, 71–78.
Hochereau-de Reviers, M.-T., Lincoln, G., 1978. Seasonal variation in the histology of the testis of the red deer, *Cervus elaphus*. J. Reprod. Fertil. 54, 209–213.
Hopkins, F.M., Spitzer, J.C., 1997. The new Society for Theriogenology breeding soundness evaluation system. Vet. Clin. North Am. Food Anim. Pract. 13, 283–293.
Houston, D.B., 1968. The Shiras moose in Jackson Hole, Wyoming. Grand Teton Nat. Hist. Assoc. Tech. Bull. 1, 110 pp.
James, A.N., Green, H., Hoffman, S., Landry, A.M., Paccamonti, D., Godke, R.A., 2002. Preservation of equine sperm stored in the epididymis at 4 °C for 24, 48, 72 and 96 hours. Theriogenology 58, 401–404.
Kenagy, G.J., Trombulak, S.C., 1986. Size and function of mammalian testes in relation to body size. J. Mammal. 67, 1–22.
Lagerlöf, N., 1934. Morphologische Untersuchungen öber Veränderungen im Spermabild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilität. Acta Pathol. Microbiol. Scand. 19 (Suppl), 254.
Lavsund, S., Nygrén, T., Solberg, E.J., 2003. Status of moose populations and challenges to moose management in Fennoscandia. Alces 39, 109–130.
Liberg, O., Johansson, A., Andersen, R., Linnell, J., 1998. Mating system, mating tactics and the function of male territoriality in roe deer. In: The European roe deer: the biology of success, first ed. Scandinavian University Press, Oslo, pp. 221–256.
Lincoln, G., 1971. The seasonal reproductive changes in the red deer stag (*Cervus elaphus*). J. Zool. 163, 105–123.
Lunstra, D.D., Ford, J.J., Echternkamp, S.E., 1978. Puberty in beef bulls: hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. J. Anim. Sci. 46, 1054–1062.
Malmsten, J., Söderquist, L., Thulin, C.-G., Gavier Widén, D., Yon, L., Hutchings, M.R., Dalin, A.-M., 2014. Reproductive characteristics of female Swedish moose (*Alces alces*) with emphasis on puberty, timing of oestrus, and mating. Acta Vet. Scand., http://dx.doi.org/10.1186/1751-0147-56-23.
Malo, A.F., Garde, J.J., Soler, A.J., García, A.J., Gomendio, M., Roldan, E.R., 2005. Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. Biol. Reprod. 72, 822–829.
Markgren, G., 1969. Reproduction of moose in Sweden. Swedish Wildl. 6, 127–286.
Martínez, A.F., Martínez-Pastor, F., Álvarez, M., Fernández-Santos, M.R., Esteso, M.C., de Paz, P., Garde, J., Anel, L., 2008. Sperm parameters on Iberian red deer: electroejaculation and post-mortem collection. Theriogenology 70, 216–226.
Martínez-Pastor, F., Guerra, C., Kaabi, M., Garcia-Macias, V., de Paz, P., Alvarez, M., Herraez, P., Anel, L., 2005. Season effect on genitalia and epididymal sperm from Iberian red deer, roe deer and Cantabrian chamois. Theriogenology 63, 1857–1875.
Miquelle, D.G., 1990. Why don't bull moose eat during the rut? Behav. Ecol. Sociobiol. 27, 145–151.
Monfort, S.L., Schwartz, C.C., Wasser, S.K., 1993. Monitoring reproduction in captive moose using urinary and fecal steroid metabolites. J. Wildl. Manag. 57, 400–407.
Morrell, J.M., Johannisson, A., Dalin, A.-M., Hammar, L., Sandebert, T., Rodriguez-Martínez, H., 2008. Sperm morphology and chromatin integrity in Swedish warmblood stallions and their relationship to pregnancy rates. Acta Vet. Scand. 50, 1–7.
Mysterud, A., Solberg, E.J., Yoccoz, N.G., 2005. Ageing and reproductive effort in male moose under variable levels of intrasexual competition. J. Anim. Ecol. 74, 742–754.
Nagy, S., Johannisson, A., Wahlsten, T., Ijäs, R., Andersson, M., Rodriguez-Martínez, H., 2013. Sperm chromatin structure and sperm morphology: Their association with fertility in AI-dairy Ayrshire sires. Theriogenology 79, 1153–1161.
Nilsen, E.B., Solberg, E.J., 2006. Patterns of hunting mortality in Norwegian moose (*Alces alces*) populations. Eur. J. Wildl. Res. 52, 153–163.
Peek, J.M., 1962. Studies of moose in the Gravelly and Snowcrest Mountains, Montana. J. Wildl. Manag. 26, 360–365.
Pinsker, W., 1978. Seasonal variations of the testicles and epididymes of red deer of different age groups before, during and after rutting time. Univerisity of Vienna, pp. 46 pp (Dissertation).
Pusenius, J., Pesonen, M., Tykkyläinen, R., Walleín, M., Huittinen, A., 2008. Hirvi- kannan koko ja vasatuotto 2006. (Moose population size and calf production in 2006). In: Wikman, M. (Ed.), Riistakannat : riistaseurantojen tulokset (Game populations 2007: results of game monitoring).
R Core team, 2013. R Foundation for Statistical Computing. In: R: A language and environment for statistical computing. R Core team, Vienna.
Sand, H., Cederlund, G., 1996. Individual and geographical variation in age at maturity in female moose (*Alces alces*). Can. J. Zool. 74, 954–964.
Sand, H., Bergström, R., Cederlund, G., Östergren, M., Stålfelt, F., 1996. Density-dependent variation in reproduction and body mass in female moose *Alces alces*. Wildl. Biol. 2, 233–245.
Schwartz, C.C., 1992. Reproductive biology of North American moose. Alces 28, 165–173.
Schwartz, C.C., 2007. Reproduction, Natality and Growth. In: Schwartz, C.C., Franzmann, A.W., McCabe, R.E. (Eds.), Ecology and management of the North American moose. , second ed. University Press of Colorado, Boulder, pp. 141–172.
Schwartz, C.C., Hundertmark, K.J., 1993. Reproductive characteristics of Alaskan moose. J. Wildl. Manag. 57, 454–468.
Schwartz, C.C., Regelin, W.L., Franzmann, A.W., 1982. Male moose successfully breed as yearlings. J. Mammal. 63, 334–335.
Short, R., 1997. The testis: the witness of the mating system, the site of mutation and the engine of desire. Acta Paediatr. 86, 3–7.
Sigouin, D., Ouellet, J., Courtois, R., 1995. Moose (*Alces alces*) rutting period variations. Alces 31, 185–197.
Solberg, E.J., Loison, A., Ringsby, T.H., Sæther, B.E., Heim, M., 2002. Biased adult sex ratio can affect fecundity in primiparous moose *Alces alces*. Wildl. Biol. 8, 117–128.
Solberg, E.J., Loison, A., Gaillard, J.M., Heim, M., 2004. Lasting effects of conditions at birth on moose body mass. Ecography 27, 677–687.
Solberg, E.J., Heim, M., Grøtan, V., Sæther, B.E., Garel, M., 2007. Annual variation in maternal age and calving date generate cohort effects in moose (*Alces alces*) body mass. Oecologia 154, 259–271.
Solberg, E.J., Sæther, B.E., Strand, O., Loison, A., 1999. Dynamics of a harvested moose population in a variable environment. J. Anim. Ecol. 68, 186–204.
Solberg, E.J., Sæther, B.E., 1994. Male traits as life-history variables: annual variation in body mass and antler size in moose (*Alces alces*). J. Mammal. 75, 1069–1079.
Soler, A.J., Esteso, M.C., Fernándes-Santos, M.R., Garde, J.J., 2005. Characteristics of Iberian red deer (*Cervus elaphus* hispanicus) spermatozoa cryopreserved at 5 °C in the epididymis for several days. Theriogenology 64, 1503–1517.
Sæther, B.-E., Haagenrud, H., 1983. Life history of the moose (Alces alces): fecundity rates in relation to age and carcass weight. J. Mammal. 64, 226–232.
Sæther, B.-E., Heim, M., 1993. Ecological correlates of individual variation in age at maturity in female moose (*Alces alces*): the effects of environmental variability. J. Anim. Ecol. 62, 482–489.
Thompson Jr., D.L., Pickett, Squires, B.W., Amann, E.L., 1979. Testicular measurements and reproductive characteristics in stallions. J. Reprod. Fertil. Suppl. 27, 13–17.
Van Ballenberghe, V., Miquelle, D.G., 1993. Mating in moose: timing behavior, and male access patterns. Can. J. Zool. 71, 1687–1690.
Van Ballenberghe, V., Miquelle, D.G., 1996. Rutting behavior of moose in central Alaska. Alces 32, 109–130.
Wallin, K., 1996. Predicting body mass from chest circumference in moose *Alces alces*. Wildl. Biol. 2, 53–58.
Williams, W.W., 1920. Technique of collecting semen for laboratory examination with a review of several diseased bulls. Cornell Vet. 10, 87–94.
Wolfe, M., 1969. Age determination in moose from cemental layers of molar teeth. J Wildl. Manag. 33, 428–431.